

The Nutritive Value of Blood-Meal Protein for Growth

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PREFACE

Blood meal is an important by-product of the meat packing industry. At present most of it is used as a fertilizer, some is used in industrial processes, and a small amount is used for animal feeding purposes. Blood meal carries a higher percentage of protein than any of the other animal protein concentrates. Unless blood meal is of low biological value, it would be more economical to use it as a feeding stuff than as a fertilizer. This investigation is a study of the biological value of blood meal proteins for growth.

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INTRODUCTION

Blood meal is an important by-product of the meat packing industry. Forty-five analyses reported by Henry and Morrison (41) give its composition as follows: 9.7 percent moisture, 3.3 percent ash, 82.3 percent crude protein, 3.8 percent nitrogen-free extract, and 0.9 percent fat. Clemen (13) lists the following uses of blood: fertilizer, animal feed, refining sugar, buttons, imitation tortoise-shell articles, therapeutic iron preparations, calico printing, finishing leather, and plaster.

In the light of present day knowledge of nutrition, the use of blood meal for fertilizer is probably not good economy. Unless the biological value of blood meal is much inferior to that of other protein materials of animal origin, it should find greater use as an animal feed.

REVIEW OF THE LITERATURE

Palatability is important in measuring the value of a feeding stuff. Kellner (47) and Lindsey (49) reported that blood meal properly prepared for animal feeding purposes was readily consumed by all farm stock. However, a number of German investigators, among them Hansen (37), Ramm (77), Goldschmidt (26), and Strohmer (86), have incorporated molasses in rations containing blood meal to improve their palatability and increase their keeping qualities. Otis (72) and Hansson (38) cautioned against feeding large quantities of dried blood in rations if they are to remain palatable for livestock. Evvard (20) fed corn and blood meal separately in self-feeders to hogs. The blood meal apparently was consumed about as well as tankage. Newman (63) and Sheehy (82) found a big difference in the palatability of blood meals, depending on their preparation. Only fresh blood, prepared before objectionable odors and decomposition set in, was regarded as suitable for animal feeding. It was tolerated by pigs after three or four days, and apparently they liked the taste. Graham (27) found that blood meal was not relished by chickens. Stammers (84) and Hoagland (42) found that purified rations containing blood meal were poorly relished by growing rats.

Digestibility is the first determining factor of the value of a feeding stuff. Wildt (93) found that samples of blood meal that had been overheated showed a 61 to 72 percent digestibility of the protein. Kellner (47) stated that blood meal, unless overheated, is an easily digested protein feeding stuff. Lindsey (48, 49), from digestion experiments with sheep, found the digestibility of the dry matter of blood meal to be 95 percent. The protein of the blood meal was 84 percent digestible. He concluded that blood meal that is not overheated is easily digested. Beck (4) reported that the nitrogen of blood sausage was well utilized. Honcamp (44) in digestion trials with sheep found the water soluble crude protein of blood meal to be 96 percent digestible, and the water insoluble crude protein 86 percent digestible. Morgan (60) reported that sheep digested 75 percent of the organic matter in blood meal. Forbes (23) found that dried-blood albumen is practically 100 percent digestible when fed to swine with corn. Caldwell (9) found that a change from liquid to dried blood in calf feeding diverts more excretory nitrogen from urine to feces. This would indicate a better digestibility of liquid blood. Brunnick (7) reported that coarse dried blood, when fed with roughage, showed a crude protein digestibility of 79 percent, while the protein of the finely ground product was 61 percent digestible. Kaupp (46) found that chickens digested 88 percent of the crude protein of blood meal.

The mineral content of blood meal is low. Blood has been used as a protein concentrate low in ash by Forbes (22), Weiser (92), and Hogan (43). Groh (34) was unable to demonstrate that the feeding of blood meal, which is comparatively rich in organic iron, to pigs increased the iron content of the body.

Protein is the chief component of blood meal. Since it carries more than 80 percent protein, blood meal has been used chiefly as a protein supplement for carbohydrate feeds. Sanborn (81), Henry (39, 40), Wilson (94), Day (18), Quick (76), Zaitschek (98), Forbes (22), Hansson (38), Hogan (43), Evvard (20), and Newman (63) have shown that blood meal supplements rations composed of cereal grains and their by-products when fed to growing pigs. Quick (76) and Sheehy (82) considered the protein of blood meal equal to that of milk in feeding value. Evvard (20) found that blood meal is of lower value than tankage. Lindsey (49) and Ellett (19) used blood meal as a protein supplement in dairy rations with success. It gave as good results as cottonseed meal. Cumming (14) and Hansson (38) fed blood meal in rations for fattening

steers with some beneficial results. Clark (12) concluded that dried blood is uneconomical as a steer feeding stuff. Carr (10), Caldwell (9), and Evvard (21) tried blood meal as a substitute for milk in rations for young growing animals, but with little success. Hogan (43), from experiments with rats, concluded that blood meal carries protein of low biological value. McCollum (51) and Hoagland (42), from experiments conducted with rats, found that blood-meal proteins are inferior to the muscle proteins. Bohstedt (6) published data from experiments with both rats and swine which show that the proteins of blood meal are of low biological value and inferior to tankage, fish meal, and casein.

Blood meal and other packing house by-products are deficient in vitamins. Bohstedt (5), from experiments with rats, concluded that blood meal carries only a trace, if any, of vitamins A and B. Stammers (84) has also shown that blood meal is deficient in vitamin B.

Otis (72) was of the opinion that blood meal serves as a tonic when fed to growing calves. Burkett (8) believed that it acted similarly when fed to work horses. Cumming (14), Hansson (38), Newman (63), and Sheehy (82) indicated that blood meal may show toxic properties unless specially prepared so as to be free from decomposition products, and they recommended that it be incorporated in rations in small quantities. Bohstedt (6) also expressed the opinion that toxic substances may be present.

A careful examination of the experimental data that have been published reveals many conflicting statements and inconclusive results concerning the nutritive value of blood meal. It appears that the nutritive value of the protein depends partly upon the process of preparation and partly upon the amino acid makeup.

This investigation was undertaken for the purpose of gaining further knowledge concerning the nutritive value of blood meal as a source of protein for growing animals. It was desired (1) to study the influence of the method of preparation, with particular reference to heat, on the digestibility of blood-meal nitrogen; (2) to determine the biological value of blood-meal protein for growth; (3) to determine some of the factors which influence the biological value of blood-meal protein; and (4) to study ways of improving the nutritive value of blood-meal protein by supplementing with other proteins.

1—DIGESTIBILITY

The first factor affecting utilization of consumed blood-meal protein is that of digestibility. Since Kellner (47) and Lindsey (49) have cautioned against the use of blood meal that has been overheated, it was decided to determine the digestibility of the product as it is generally put on the market at the present time. According to Aldrich (1) commercial blood meal is prepared in an open tank with live steam while stirring by mechanical agitators to secure thoro coagulation of the protein. After cooking about 30 minutes, the material is allowed to settle and the lower, or liquid, portion is drained off. Some plants make a practice of evaporating this blood water, but it is seldom economical because of the low solid content. Blood water is also somewhat hard on the evaporators, as it, more than other cooking waters, has a tendency to corrode metal. After draining, the coagulated blood is gravitated directly to the press and pressed while still hot. After pressing, the product contains about 45 percent moisture. The material is then placed in steam jacketed driers, stirred by paddle agitators, and dried at a temperature of 150 to 175° C (302 to 347° F). The coagulated dried blood is then ground and put thru a 40-mesh screen.

Two different blood-meal products were prepared for the determination of digestibility as follows: 150 gallons of fresh hog blood was dried at room temperature by placing in shallow pans and directing a continuous current of air over it by means of electric fans. The dried material was removed from the pans and ground to pass a 40-mesh sieve. One-half of the air-dried blood was cooked one hour in a large open iron kettle with distilled water and constant stirring. The resulting product was pressed while still hot to remove the cooking water. Then the coagulated sausage-like material was placed in shallow pans and dried from 24 to 48 hours in a hot air oven at 100 to 120° C (212 to 248° F). The dried lumpy material was then ground to pass a 40-mesh sieve. A third sample of blood meal used was a commercial product typical of what is sold as blood meal for animal feeding purposes.

Pigs and rats were used as experimental animals. Pigs were used because blood meal as an animal feed finds its chief use as a supplement to corn in pig feeding. Rats were used for comparison, since a further study of blood meal was to be made by using rats, and because the question is often raised of how far results obtained with small animals, as rats, are comparable to results obtained with

domestic animals. Because blood meal is too rich in protein to be fed alone, its digestibility was found by difference between the digestibility of a basal ration of corn and that of the same basal ration plus blood meal. Nitrogen balances were determined along with the digestibility of the nitrogen of the rations, for this involved very little more labor and at the same time furnished information concerning the metabolism of absorbed nitrogen.

EXPERIMENTS WITH PIGS

Experimental

Four Tamworth pigs, two males and two females, weighing approximately 80 pounds were used. They were confined in metabolism crates, described in Ohio Experiment Station Bulletin 271. The feed consisted of corn 75 parts, blood meal 25 parts, and 1 part sodium chloride to 200 parts of the mixed concentrate. The feed for all the periods was prepared at one time, sampled for analysis, and individual feeds weighed into paper sacks and stored until time of feeding. The pigs were fed 900 grams of feed twice a day, the feed being mixed with sufficient distilled water to make a thin slop. Additional distilled water was furnished ad libitum during the day. A preliminary period lasting 7 days preceded each collection period of 10 days. The beginning and ending of periods were designated by time instead of markers. The crates were cleaned every other day. The feces were removed from the screens by wire brushes and brushed from the sheets into tarred 5-gallon lard cans. The sheets and cages were washed with cold distilled water. The wash water was filtered thru cheese cloth and added to the urine. Any residue remaining on the filter cloth was transferred to the feces can.

In each urine bottle were placed 5 cc. of a 10 percent solution of thymol in chloroform to insure the preservation of the urine, and 5 cc. of glacial acetic acid to prevent the possible precipitation of salts. The urine bottles were renewed each morning and evening, the collected urine being placed in cold storage at a temperature of about 2° C (36° F) until the crates were cleaned. The combined washings and urine were then measured, placed in a large container, thoroly shaken, and an aliquot of one-tenth the total saved in a sample bottle for analysis. The urine samples for analysis were kept in cold storage at 2° C until ready for use.

The feces were kept in cold storage below freezing temperature. They were weighed and then ground thru a sausage mill

while frozen, thus giving a more uniform sample for analysis. Samples were taken for analysis, placed in weighing bottles, and returned to cold storage until time for analysis.

The chemical analyses were made in accordance with the Methods of Analysis of the Association of Official Agricultural Chemists.

Discussion

The general scope of the pig experiment and plan of feeding are given in Table 1. Data concerning the digestion and metabolism of nitrogen are summarized in Table 2. The pigs showed no dislike for the rations, and feed was reduced in only two or three instances. Very little feed was scattered, and as much of it as possible was returned to the feed box. At the end of the periods no feed was left.

TABLE 1.—Plan of Feeding Rations to Pigs

Pig No.	Period I	Period II	Period III	Period IV
1	Corn	Corn and air-dried pig blood	Corn and cooked pig blood	Corn and commercial blood meal
2	Corn	Corn and air-dried pig blood	Corn and cooked pig blood	Corn and commercial blood meal
3	Corn and air-dried pig blood	Corn	Corn and cooked pig blood	Corn and commercial blood meal
4	Corn and air-dried pig blood	Corn	Corn and cooked pig blood	Corn and commercial blood meal

The order in which the corn ration and corn-plus-blood-meal rations were fed made no apparent difference in the digestibility of the rations. As far as the metabolism of nitrogen is concerned, it would seem that feeding the corn plus blood meal first caused greater excretion of nitrogen in the urine on the subsequent corn ration, which in turn showed a smaller percentage of digestible nitrogen retained. In other words, there was some evidence that the order in which the rations were fed made a difference of about 4 percent in the results obtained. During the period on the corn ration the body may have been partially depleted of readily available nitrogen to make up for the inefficiency of the corn protein. Then when the change was made to the corn-plus-blood-meal rations there was probably increased storage to make up for the previous losses. After the period on the corn-plus-blood-meal rations there would probably be excessive storage of nitrogen so that there would not be such economical use of the nitrogen of the corn ration. The same line of reasoning would then account for the better utilization of absorbed nitrogen by Pigs 3 and 4 in Period 3.

TABLE 2.—Apparent Digestibility and Metabolism of Nitrogen of Feeds by Pigs (10 days)

Ration	Pigs		Feed intake		Nitrogen content of feces	Apparent digestibility of ration	Nitrogen content of urine	Utilization		Digestibility of blood meal
	Number	Gain in weight	Weight	Nitrogen content				Balance	Absorbed nitrogen retained	
Corn	1	<i>Kg.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Pct.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Pct.</i>	<i>Pct.</i>
	4		18,000	266.13	58.79	77.91	141.77	65.57	31.62
	2	6	18,000	266.13	61.63	76.84	148.84	55.66	27.22
	3	5	18,000	266.13	45.61	82.86	166.27	54.25	24.60
	4	4	18,000	266.13	59.32	77.71	157.09	49.72	24.04
	A v.					78.83			26.87
Corn and air-dried blood	1	9	18,000	812.50	57.99	92.86	511.38	243.14	32.22	97.46
	2	7	18,000	812.50	77.02	90.52	438.54	236.94	32.22	94.34
	3	5	18,000	812.50	84.39	89.61	538.99	189.12	25.97	90.82
	4	5	18,000	650.00	67.37	89.63	410.92	171.70	29.47	92.83
	A v.					90.65			29.97	93.86
Corn and cooked blood	1	6	18,000	837.38	147.78	82.35	553.58	136.02	19.72	81.85
	2	7	17,000	795.51	132.39	83.36	478.00	185.12	27.92	83.70
	3	8	18,000	837.38	123.46	85.26	502.11	211.81	29.67	84.38
	4	8	17,000	795.51	147.45	81.46	472.24	175.81	27.13	87.60
	A v.					83.11			26.11	84.38
Corn and commercial blood	1	4	18,000	773.95	189.00	75.58	471.89	113.06	19.33	71.46
	2	5	18,000	773.95	193.42	75.01	432.83	147.70	25.44	71.01
	3	5	18,000	773.95	194.03	74.93	460.02	119.90	20.68	68.53
	4	7	18,000	773.95	187.72	75.77	432.19	154.24	26.30	78.85
	A v.					75.32			22.94	72.46

The apparent digestibility of the nitrogen of the rations decreased in the order: corn and air-dried blood, corn and cooked blood, and corn and commercial blood. From calculations of digestibility of blood meal by difference, Table 3 shows that digestibility is decreased as the temperature used in the method of preparation is increased.

TABLE 3.—Digestibility of Air-dried, Cooked, and Commercial Blood Meal

Sample	Temperature of drying	Digestibility
	<i>Degrees C.</i>	<i>Pct.</i>
Air-dried blood	30	94
Cooked blood	115	84
Commercial blood	175	72

It is probable that the error, due to associative digestibility, just about equals the error due to products of metabolic origin, and that these figures give a fair average of the true digestibility. A striking example of associative digestibility is the effect of milk on the digestibility of the proteins of corn, as shown in an experiment by Hart (35) on swine. As the percentage of milk proteins was increased from 3.2 percent to 28.5 percent of the total protein in the ration, the co-efficient of digestibility of the protein mixture increased from 55 to 83, and of the corn protein from 49 to 77, assuming complete digestibility of the milk proteins. Forbes (23) secured data indicating that when the fecal nitrogen of metabolic origin is not deducted in digestion experiments with swine, the digestibility of protein is under-estimated by about 20 percent.

Some interesting results are shown in the data on the metabolism of nitrogen (Table 2). As the digestibility decreased the amount of nitrogen excreted in the urine did not decrease in proportion, thereby causing a decrease in the percentage of absorbed nitrogen retained.

It seems that two factors may account for this condition. First, blood meal may contain unknown substances which stimulate protein catabolism. Second, drying at a high temperature may have broken up the blood-meal proteins into nitrogenous fragments of low biological value, in which case the urine would be the natural path of elimination of the digestible fractions.

If the biological values of the proteins of corn and blood meal were the same, when the amount absorbed was increased, there would be a corresponding decrease in the percentage of absorbed nitrogen retained because, according to Mitchell (58), increasing the quantity of amino acids decreases the efficiency of utilization.

On the contrary, with the exception of commercial blood meal that had been heated to a relatively high temperature, the percentage of absorbed nitrogen retained remained the same or increased. Therefore, the percentage of absorbed nitrogen retained indicates a favorable supplementary action between the proteins of corn and blood meal, and a difference in the biological value of the different blood-meal preparations.

EXPERIMENTS WITH RATS

Experimental

The method of procedure used in the metabolism studies with rats was in general principle the same as that described by Mitchell (57, 59). Three albino rats, one male and two females, averaging about 110 grams in weight, were used for the work. In the selection, it was desired to secure rats that were in the same stage of their life cycle as the pigs. The rations fed were those fed to the pigs during the first three periods. The rations were fed in the following order: corn, corn plus air-dried blood, and corn plus cooked blood. Another group of three rats was used for determining the digestibility of the corn-plus-commercial-blood-meal ration.

Discussion

Data concerning the digestibility and metabolism of the nitrogen of the rations are presented in Table 4. The nitrogen of the air-dried-blood ration was more digestible than the corn-plus-cooked-blood ration, the latter being about as well digested as the corn ration alone. The commercial blood meal was less digestible than the cooked blood. The digestibility of the corn ration was about the same for both groups of rats.

There was better retention of corn nitrogen than of that in the mixture of corn and blood meal. The mixture, no doubt, furnished such an abundance of nitrogenous material that the cells were unable to metabolize it economically. However, Steck (85) has shown that hemoglobin tends to increase urinary nitrogen, and may be a causative factor here. The second group of rats made better use of the absorbed nitrogen than the first group. This was very likely due to the fact that the second group had previously been on a nitrogen-free diet. Probably the readily available nitrogen of the tissues was more depleted and the rats accordingly would make more economical use of the absorbed nitrogen.

TABLE 4.—Apparent Digestibility and Metabolism of Nitrogen of Rations by Rats (7 days)

Ration	Rats		Food		Nitrogen content of feces	Apparent digestibility of rations	Nitrogen content of urine	Utilization		Digestibility of blood meal nitrogen
	Number	Gain in weight	Amount consumed	Nitrogen content				Balance	Absorbed nitrogen retained	
		<i>Gm.</i>	<i>Gm.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Pct.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Pct.</i>	<i>Pct.</i>
Corn	1	5	89.58	1324.40	293.70	77.82	787.60	243.10	23.59
	2	—2	73.06	1080.20	206.80	80.86	681.70	191.70	21.95
	3	6	74.35	1099.30	186.00	83.08	665.60	247.70	27.12
	Av.	3	79.00	1168.00	228.80	80.59	711.63	227.50	24.22
Corn and air-dried blood meal	1	9	87.53	3951.00	372.40	90.57	2671.50	907.10	25.35	94.28
	2	—5	58.60	2645.10	231.20	91.26	1973.70	440.20	18.24	95.72
	3	—14	60.00	2708.30	226.10	91.65	2231.60	250.60	10.10	95.24
	Av.	—3	68.71	3101.50	276.60	91.16	2292.30	532.60	17.90	95.08
Corn and cooked blood meal	1	10	103.14	4799.10	990.60	79.36	3030.70	777.80	20.42	77.48
	2	1	90.67	4218.10	890.30	78.89	2756.70	571.10	17.16	75.75
	3	4	81.72	3801.70	744.70	80.41	2586.30	470.10	15.40	77.20
	Av.	5	91.84	4273.00	875.20	79.55	2791.20	606.30	17.66	76.81
Corn and commercial blood meal	4	21	72.82	3131.04	741.00	76.33	1649.00	741.04	31.01	70.90
	5	24	73.66	3167.16	720.25	77.26	1663.00	783.91	32.04	72.65
	6	14	79.10	3401.06	812.50	76.11	2128.00	460.56	17.79	71.19
	Av.	20	75.19	3233.00	757.92	76.57	1813.33	661.84	26.95	71.58
Corn	4	10	98.80	1460.76	259.00	82.27	820.00	381.76	31.77
	5	8	94.04	1390.38	259.00	81.37	722.00	409.38	36.18
	6	7	99.63	1473.03	284.50	80.69	827.00	361.53	30.42
	Av.	8	97.49	1441.39	267.50	81.44	789.70	384.22	32.79

Similarity of the Metabolism of Protein by Pigs and Rats

Any consideration of the general significance of the biological values of proteins to be discussed in the succeeding part of this paper must include the question whether pigs and rats exhibit different efficiencies in their utilization of protein in anabolism. A comparison of the digestion and metabolism of the nitrogen of the rations by rats and pigs is given in Table 5. The rations were digested equally well by both species of animals. There seemed to be no appreciable difference in the utilization of the proteins of corn by pigs and rats. Owing to previous treatment, the second group of rats utilized corn nitrogen about 8 percent better than the first group. Consequently, under normal conditions, one would expect a retention of about 19 percent of the absorbed nitrogen of the commercial blood-meal ration instead of 27 percent as shown in the table. It appears that the pigs made better use of the nitrogen of the blood-meal rations than was made by the rats. This possibly may be explained by a difference in the abundance of supply.

TABLE 5.—A Comparison of the Digestibility and Metabolism of Nitrogen of Rations by Pigs and Rats

Ration	Animal	Digestibility of ration	Digestibility of blood meal	Absorbed nitrogen retained
		<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
Corn	Pigs	78.83	26.87
	Rats	81.01	28.49
	Av.	79.92	27.68
Corn and air-dried blood meal	Pigs	90.65	93.86	29.97
	Rats	91.16	95.08	17.90
	Av.	90.90	94.47	23.93
Corn and cooked blood meal	Pigs	83.11	84.39	26.11
	Rats	79.55	76.81	17.66
	Av.	81.33	80.59	21.88
Corn and commercial blood meal	Pigs	75.32	72.46	22.94
	Rats	76.57	71.58	26.95
	Av.	75.94	72.02	24.94

According to McCollum (50) the growth impetus of the pig is much greater than that of the rat, yet, according to Hogan (43), the rat eats much more food per unit of body weight for energy metabolism. Under such conditions the rat has a greater supply of nitrogen for equivalent anabolic processes, and Mitchell (58) has secured data indicating that the economy with which the cells utilize the amino acids supplied to them decreases with an increase in the supply.

The twenty-six comparisons afforded by this work indicate that the general metabolism processes of the pig and rat for nitrogen are very much alike. Differences of a quantitative nature only were shown. Data assembled by Mitchell (56) agree.

Factors Influencing the Digestibility of Blood-Meal Nitrogen

The influence of associative digestibility and nitrogen of metabolic origin have been touched upon. Forbes (23) has shown that blood meal has a marked influence on the digestibility of the proteins of corn. A theory has been advanced that concentrated protein feeds stimulate the bacteria of the digestive tract causing them to attack the carbohydrate feeds, thus disintegrating them better and freeing more of the protein which they contain. The digestibility of the rations given in Table 2 is satisfactory for comparison, but would not represent the true digestibility of the rations where the feeding is done on a more practical and economical basis. In such a case the protein concentrate, if highly digestible and of good biological value, would not constitute more than 10 percent of the ration. In agreement with Grindley (33), it appears to me that in the future the digestibility of each ration for a species of animals should be determined in the form and proportion in which it is to be fed.

The preceding work with rats and pigs confirms the report of Wildt (93), Kellner (47), and Lindsey (49), namely, that subjecting blood meal to high temperature lowers its digestibility. Forster (24), Atwater (2), Prausnitz (75), Grindley (28, 29, 30), Richert (79), Talarico (87), Mendel (53), and Wright (95) have shown that drying or cooking reduces the digestibility of meat proteins or extractives. Prausnitz (75) is of the opinion that the protein material does not remain in the digestive tract long enough for the dried particles to become sufficiently hydrated to permit the digestive enzymes to operate. It was shown by Mann (52) that when a protein coagulates an insoluble network or meshwork is formed in the interstices of which the solvent of the colloid is held. It may be assumed that the formation of globulin granules, acting as fixed points thruout the protein material, causes the particles of coagulated albumin to be more finely and evenly distributed and makes it impossible for the albumin to form loose or coarse trabeculae. Then, according to Frank (25), the digestibility decreases with increased temperature owing to increased hardness or compactness of the coagulated material. Similar methods of reasoning might be applicable to blood since it contains proteins coagulable at different temperatures.

Chick and Martin (11) have shown that heat coagulation of proteins consists of denaturation and agglutination. Denaturation, or the change from soluble to insoluble form, is regarded as a hydrolysis by Chick (11) and Wu (96, 97), and as a dehydration by Robertson (80).

Wu (97) furnished evidence which indicates that the fundamental reaction underlying denaturation is one of hydrolysis involving some specially labile linkages. It may be brought about by dilute acids and alkalis, and even more readily by heat. The denatured protein differs only slightly from the original in amino acid distribution. Some amino acids which react with Millon's reagent, probably tyrosine and tryptophane, have been shown to split off very readily. Probably other amino acids are liberated when a protein is denatured. If blood is coagulated by heat, there is more non-protein nitrogen found in the filtrate than when the coagulation takes place in the cold by means of protein precipitants in faintly acid solution.

Proteins vary in their resistance to the action of trypsin. To explain this behavior the existence of a specific anti-enzyme, anti-trypsin, has been postulated, which is supposed to unite with trypsin much as an antitoxin does with its corresponding toxin. Anti-trypsin is present in blood serum and has been extracted with chloroform by Joblin and Peterson (45). Altho this particular enzyme would be destroyed at the temperature employed in preparing blood meal, it is not impossible that other products of a similar prohibitive nature might be formed.

Bateman (3) has shown that heating changes the chemical nature of egg proteins and increases their digestibility. Proteins need only undergo slight chemical change to cause a partial reversal of their rotation of polarized light. In some instances the slight change may suffice to convert them into poorly digestible substances. Thus Dakin (15) found that racemized casein was indigestible.

2—VALUE FOR GROWTH

GROWTH STUDIES

In determining the nutritive value of blood-meal protein for growth in rats, it was desired (1) to compare different brands of commercial blood meal; (2) to compare blood meal from different species of animals; (3) to compare blood meal prepared by different processes, and (4) to compare clot and serum.

EXPERIMENTAL PLAN

The numerical method devised by Osborne, Mendel, and Ferry (70) was adopted, since it appeared to be the most suitable for the investigation. The method involves the use of control experiments and interpretation of results with reference to the amount of protein consumed, as well as the level of intake.

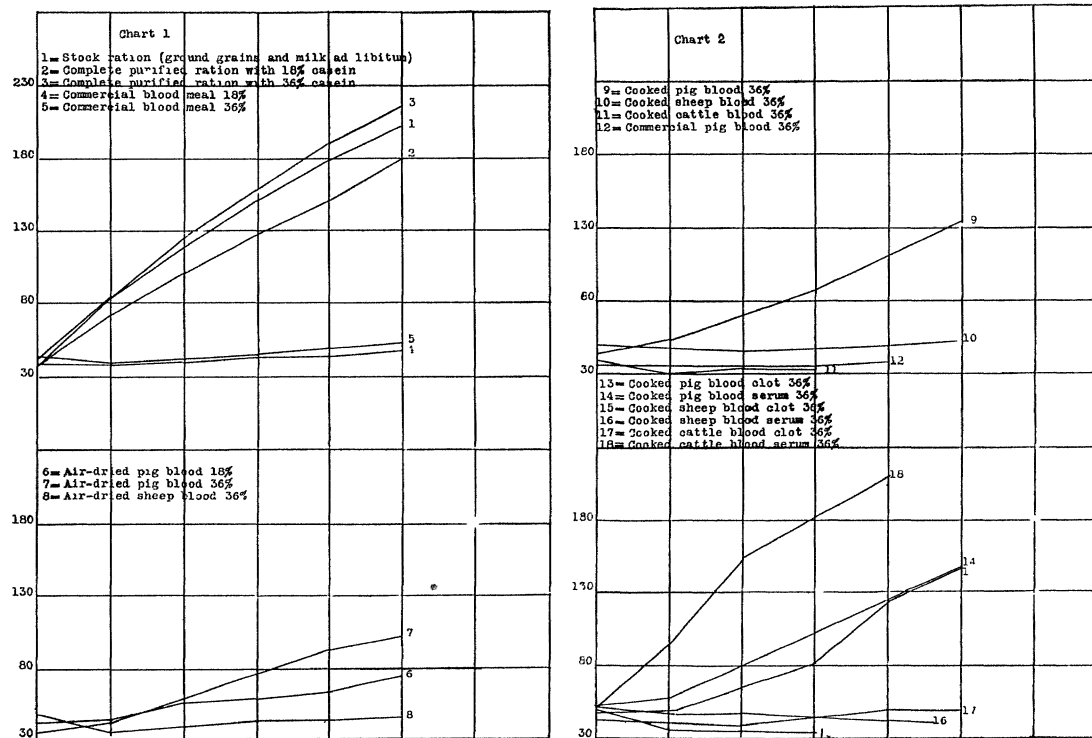
Generally accepted standard methods of procedure with small animals in nutritional investigations were followed. All the rats used came from the same stock colony and from litters which were reduced to six at birth. The age, size, and previous treatment of all rats placed on the experimental rations were approximately the same. Each lot was selected, as far as possible, to contain four rats, two males and two females, each taken from a different litter.

The complete synthetic ration adopted for the work consisted of purified casein 18, agar 2, cod-liver oil 2, crisco 5, dried brewers' yeast 6, starch 63, and the McCollum (51) No. 185 salt mixture 4. In the experimental rations the blood-meal products under investigation were substituted for the casein as the source of protein. As the proportion of blood meal varied from 18 percent, the quantity of starch was varied so as to maintain the total parts of the ration on the basis of 100.

The rats were weighed at a definite time each week. Feed consumption records were kept for each lot during the entire experimental period. The results as regards gain in weight and feed consumption are based on the average of the four individuals in a lot.

The blood meal samples used were prepared as follows: Air-dried blood meal was made by drying fresh blood in shallow pans at room temperature by means of air currents produced by electric fans. Cooked blood was made by coagulating fresh blood with live steam in an open tank for about 30 minutes, draining free of cooking water and non-coagulable material and drying in a hot-air oven at about 110° C (230° F). Clot and serum were prepared by allowing fresh blood to clot and then separating the serum from the clot by filtering thru cheese cloth. Both fractions were then cooked with live steam for about 30 minutes and dried at 110° C. All the dried products were ground to a meal which passed a 40-mesh sieve.

The general scope of the blood products fed, together with the food intake and gains, are given in Table 6, while some of the outstanding results are shown graphically in Charts 1 and 2. The complete purified control diet was adequate for normal growth, as shown by comparison with gains made with rats from the stock colony (Chart 1). Increasing the level of casein increased the rate of gain but lowered the gain per gram of protein consumed. Experimental tests with two brands of dried brewers' yeast indicated that normal growth was not secured with less than 6 percent incorporated in the diet. In some cases the nitrogen from the



Charts 1 and 2 showing value of blood meal from different species of animals and methods of preparation for growth of rats. Vertical spaces represent weight in grams, and each horizontal space represents two weeks experimental period

TABLE 6.—Nutritive Value of Blood Meal Proteins for Growth of Rats

Blood meal product	Level of intake	Lot receiving diet	Duration on experimental diet	Average daily food consumption	Gain in body weight	Total intake		Intake per gm. of gain		Gain per gm. of food	Gain per gm. of protein
						Food	Protein	Food	Protein		
	<i>Pct.</i>	<i>No.</i>	<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Casein (control ration)	18	60	110	10.3	196	1136.8	229.2	5.8	1.2	0.17	0.83
	36	41	100	10.5	225	1054.2	402.2	4.7	1.8	.21	.56
Cleveland Provision Co. commercial blood meal	18	3	119	5.3	19	636.5	128.3	33.5	6.2	.03	.15
	27	17	82	2.0	6	168.0	49.0	28.0	8.2	.04	.12
	36	43	110	6.5	37	714.1	272.5	19.3	7.4	.05	.14
Wilson & Co. spray process blood meal	18	91	62	3.4	—3	212.5	42.8
	36	92	62	3.6	0	220.0	84.0
Armour & Co. blood meal	18	94	49	4.4	—5	216.2	43.6
Air-dried pig blood	18	35	96	7.2	50	695.0	140.1	13.9	2.8	.07	.36
	27	46	110	8.5	68	931.6	271.7	13.7	4.0	.07	.25
	36	68	98	9.3	81	915.3	349.3	11.3	4.3	.09	.23
Air-dried sheep blood	18	39	114	6.2	8	710.4	143.2	88.8	17.9	.01	.06
	27	21	112	4.6	16	512.0	149.3	32.0	9.3	.03	.11
	36	48	110	4.7	7	512.2	195.5	73.2	27.9	.01	.04
Cooked pig blood	18	37	114	8.0	49	916.3	184.7	18.7	3.8	.05	.26
	27	47	110	8.3	103	916.7	267.3	8.9	2.6	.11	.38
	36	69	98	8.1	122	793.0	302.6	6.5	2.5	.15	.40
Cooked sheep blood	18	61	40	3.0	—11	121.5	24.5
	27	22	112	6.6	24	734.4	214.2	30.6	8.9	.03	.11
	36	49	103	7.3	21	753.9	287.7	35.9	13.7	.03	.07
Cooked cattle blood	18	55	54	1.9	—10	105.0	21.2
	27	76	77	5.4	20	414.0	120.7	20.7	6.0	.05	.17
	36	57	47	3.5	—8	166.7	63.6

TABLE 6.—Nutritive Value of Blood Meal Proteins for Growth of Rats—Continued

Blood meal product	Level of intake	Lot receiving diet	Duration on experimental diet	Average daily food consumption	Gain in body weight	Total intake		Intake per gm. of gain		Gain per gm. of food	Gain per gm. of protein
						Food	Protein	Food	Protein		
	<i>Pct.</i>	<i>No.</i>	<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Cleveland Provision Co. commercial pig blood	18	89	49	4.6	—11	226.2	45.6
	27	30	112	5.0	23	563.7	164.4	24.7	7.1	0.04	0.14
	36	44	47	8.1	—1	379.0	144.6
Cooked pig blood heated to 145° C.	36	84	56	6.8	—11	379.0	144.6
Pig blood dried at 110° C.	18	38	114	7.8	20	890.2	179.5	44.5	9.0	.02	.11
	36	72	63	6.5	9	411.2	156.9	45.7	17.3	.02	.06
Pig blood dried at 80° C.	18	90	70	5.8	11	403.7	81.4	36.7	7.4	.03	.14
Sheep blood dried at 110° C.	36	80	91	6.0	13	547.5	208.9	42.1	16.1	.02	.06
Cooked pig blood clot	27	14	103	9.3	89	961.2	280.3	10.8	3.1	.09	.32
	36	77	77	10.6	95	818.7	321.4	8.6	3.3	.12	.30
Cooked pig blood serum	27	15	103	8.9	74	916.7	267.3	12.4	3.6	.08	.03
	36	70	98	9.4	133	918.7	350.6	6.9	2.6	.14	.38
Cooked sheep blood clot	27	24	35	—10
	36	78	42	4.5	—17	187.5	71.6
Cooked sheep blood serum	18	63	28	4.6	—6	130.0	26.2
	27	31	105	9.3	166	980.0	285.8	5.9	1.7	.17	.59
	36	74	63	6.7	—10	420.0	160.3
Cooked cattle blood clot	18	96	75	5.1	—5	383.7	77.4
	36	79	77	5.0	0	388.7	148.3
Cooked cattle blood serum	18	97	75	5.6	7	420.0	84.7	60.0	12.1	.02	.08
	36	75	49	11.4	159	557.5	212.7	3.5	1.3	.29	.77

yeast and agar furnished as high as 12 percent of the total nitrogen intake, but since these constituents were kept constant in all the rations, the results have a comparative, if not an absolute, value.

The blood-meal rations varied greatly in palatability. In most cases they were not liked by young rats. Increasing the percent of blood meal in the ration resulted in a little better feed consumption in most cases. This would tend to disprove the idea of toxic substances in blood meal, for, if they were present, increasing the percent of blood meal in the rations would increase the harmful effects.

The method of preparing blood meal had a great influence on palatability. Commercial blood meal, which had been heated to a high temperature, was unpalatable. Where such products were used in rations the feed intake was so low that practically no growth was obtained. Air-dried and cooked blood were about equal in palatability and superior to commercial blood meal (Chart 1). Some growth was secured where they were used. Blood serum appeared to have greater nutritive value than blood clot. The marked variations in results obtained in some instances with the same product in different lots may be explained by the fact that all lots were not on experiment at the same time, and the material for the different lots was not made at the same time. The time of cooking the blood or temperature of drying may not have been the same in all cases. In one or two cases, it was found that decomposition had started before the product was thoroly dry. The good results obtained with Lots 31 and 75 indicate the possibility of preparing blood products which have a high nutritive value.

Pig blood was more palatable than cattle or sheep blood. In most cases good feed consumption was secured and fair growth obtained. There is some indication that the blood of omnivora is better for rats than the blood of herbivora (Charts 1 and 2). Such differences may be expected from the characteristics of the blood of different species, as shown by Reichert (78). In omnivora the percentage of albumins is greater than that of the globulins, while in herbivora the reverse is true. There is a great difference in the solubility of the fibrins of different species, that of the pigs being most soluble and of cattle least soluble. Another important means of biological differentiation has been brought to light during recent years in the discovery of zooprecipitins and physiological reactions pertaining to the chemistry of immune serums. The results of the investigations in this field point clearly to the specificity of certain,

as yet obscure but closely related, proteins to certain blood constituents of different genera. Commercial blood meal generally consists of blood of both omnivora and herbivora, with the latter predominating. This may be one of the reasons accounting for the poor results secured by feeding commercial blood meal to rats. The same explanation may partly account for the better use made of pig blood than of commercial blood by pigs, and of the less efficient use by rats as shown by the nitrogen metabolism studies in the preceding part of this work. In other words, it is believed that blood meal made from one species of animals possesses greater nutritive value for animals of that species than the blood meal from any other species.

The results obtained from thirty feeding trials with blood meals from three sources and five methods of preparation showed that the products vary in palatability. Because of difference in food intake in the various lots and length of time of the feeding trials, it was hard to make direct comparisons. In all cases the food intake was more than sufficient to meet the calorific requirement for maintenance. Where whole blood meal rations were well consumed, as in Lots 44, 46, 47, and 68, growth was not nearly as good as where casein was used. Therefore, it appears that blood meal as a feeding stuff is not only unpalatable, but carries proteins of low biological value for growth.

METABOLISM STUDIES

The gain in weight of an animal on a ration presumably deficient in protein only is a somewhat uncertain indication of protein retention, since deposited material is of variable composition and since gains may be limited by deficiencies in growth determinants at present unknown. Growth studies will show differences in protein, but will not explain why one protein is better than another. Valuable aids to the solution are metabolism studies and chemical analyses of the protein. By metabolism studies one may determine differences in the intestinal utilization of dietary nitrogen. By indirect methods one may determine the actually absorbed nitrogen, due allowance being made for nitrogen of body or bacterial origin excreted in the feces and urinary nitrogen of body origin.

The biological values of the proteins of a number of blood-meal rations, similar to those fed in the preceding work in growth studies, were determined according to the method used by Mitchell (57, 59). The nitrogen-free diet used was the standard purified

control diet minus agar, yeast, and protein. The general scope of the nitrogen metabolism studies and the order in which the diets were fed are given in Table 7. The results of the work are given in Table 8. The results for fecal and urinary nitrogen of body origin used in the calculations are based on the average of the two periods of nitrogen-free feeding.

TABLE 7.—Plan of Feeding Rations for Metabolism Studies with Rats

Period	Diet—Rats 1—5	Diet—Rats 6—10
1	Nitrogen-free	Nitrogen-free
2	Air-dried pig blood	Air-dried sheep blood
3	Commercial pig blood	Cleveland Provision Co. blood meal
4	Wilson & Co. blood meal	Wilson & Co. blood meal 15 percent and casein 3 percent
5	Casein	Armour & Co. blood meal
6	Cattle clot	Cattle serum
7	Nitrogen-free	Nitrogen-free

Discussion

There was considerable variation in the metabolic nitrogen of the feces of different rats, and no marked difference from the first results was noticed during a subsequent period of non-protein feeding twelve weeks later. The endogenous nitrogen also varied for the different rats. During the 12-week interval between feeding trials on the nitrogen-free diets there was a marked decrease in the endogenous nitrogen.

The biological value of casein fed at 18 percent level was low. This may be accounted for because of the relatively high percentage of good protein in the diet, and the large quantity eaten. The greater the quantity of nitrogen absorbed the less efficient use was made of the material. This was probably due in part to a lower utilization for growth than for maintenance, and to the increasing ratio of the absorbed nitrogen used for growth to that used for maintenance as the rate of growth increases. Also, the protein material absorbed in excess of that which could be used for synthetic purposes would be oxidized by the processes of exogenous protein metabolism.

The biological value of the commercial brands of blood meal, when determined by metabolism experiments, decreased in the same order as when determined by growth experiments. The sheep blood indicated poorer results when determined by the

TABLE 8.—Biological Value of Blood Products at an 18 Percent Intake (Average for 7 days)

Distinguishing feature of ration	No. of rats used	Average weight of rats	Food con- sumed	Nitrogen intake	Fecal nitrogen	Apparent digestibility	Metabolic nitrogen of feces	Food nitrogen in feces	Urinary nitrogen	Endogenous nitrogen	Food nitrogen in urine	Biological value
		<i>Gm.</i>	<i>Gm.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Pct.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Pct.</i>
Nitrogen-free	10	84	47.3	36.39	87.10	(1.84)	207.60	(2.47)
Cleveland provision Co.	5	86	32.7	1080.81	311.81	71.17	54.61	257.40	660.00	166.84	493.16	40
Armour & Co.	5	112	49.67	1486.57	511.15	65.62	82.95	428.20	915.20	217.28	697.92	34
Wilson & Co.	5	91	45.27	1408.41	239.95	82.96	75.60	164.35	1186.30	176.54	1009.76	19
Wilson & Co. 15 percent and casein 3%...	5	91	40.16	1266.12	220.05	82.62	67.07	152.98	821.20	176.54	644.66	42
Casein	5	147	69.88	2151.67	249.05	88.43	116.70	132.35	1527.50	285.18	1242.32	38
Air-dried pig blood	5	81	57.25	1652.12	255.90	84.51	95.61	160.29	1220.20	157.14	1063.06	29
Air-dried sheep blood	4	78	21.95	674.38	141.37	79.04	36.66	104.71	1006.62	151.32	855.30	—50
Commercial pig blood	5	101	69.83	2359.40	1745.40	26.02	116.62	1628.78	553.00	195.94	377.06	54
Cattle clot	5	140	28.57	951.90	223.60	76.51	47.71	175.89	826.00	271.60	554.40	29
Cattle serum	5	110	38.82	1321.50	343.05	74.04	64.83	278.22	688.60	213.40	475.20	55
Nitrogen-free	10	119	40.00	22.78	60.52	(1.51)	167.40	(1.41)

metabolism method than when determined by the growth method. Casein showed a marked supplementary effect on blood meal. The spray-process blood meal was about as well digested as air-dried blood. Pig blood had a higher biological value than cattle blood (spray process) or sheep blood. Commercial pig blood had a higher biological value than air-dried pig blood, altho it was much less digestible. The difference may be explained in part by a difference in quantity of available nitrogen present. Altho there was little difference in the digestibility of clot and serum, the latter had a much higher biological value.

The determination of the biological value of a protein by Mitchell's method is not without faults. There are many opportunities for errors in the metabolism procedure. The method for determining the excreted nitrogen, due to body origin, also may be justly criticised. Among other things, it fails to consider the influence of a medium level of intake of protein on the bacteria of the intestinal flora. The determination of the biological value of a protein by this method may serve a useful purpose in checking results by growth studies. It gives results which agree with those obtained by growth studies, but makes them stand out more prominently. A combination of the numerical and biological methods for determining the nutritive value of a protein seems to be the most reliable method in use at the present time.

3—SOME LIMITING FACTORS

EXTRACTIVES

Blood carries a large variety of organic substances and these are so prone to chemical change that there may be originally present in blood, or developed in the process of making blood meal, constituents of low or even harmful nutritive value. Mitchell (59) reported data indicating that the nitrogenous extractives of meat are of low biological value, and that the nutritive value of meat is improved by their removal.

It was desired to find whether the nutritive value of blood meal would be improved by the removal of some of the extractives. A sample of commercial pig blood that had shown a very low biological value for growth was placed on a large Buchner funnel and extracted with 200 times the amount of cold distilled water added in small quantities at a time. Other samples were similarly extracted by the use of hot water, 95 percent cold alcohol, and ether. A sample of commercially mixed blood was extracted by shaking with alcohol followed by ether, and another with alcohol

followed by hot water. A sample of Wilson & Company's blood meal, manufactured by a spray process, requiring a relatively low temperature, was dried and extracted 48 hours in Soxhlet extractors with anhydrous ether. Another sample of the same product was similarly extracted with absolute alcohol.

The results of the use of the extracted products as the only source of protein for growth of rats are given in Table 9. Either hot or cold water removed an appreciable quantity of material, but no difference in the nutritive value of the extracted and unextracted products was noticed. Alcohol and ether removed very little material from the commercial pig blood. However, each solvent must have removed material of low biological value from commercially mixed blood, for, by comparing the growth of Lot 43 with Lots 50 and 51, the extracted material produced more growth, and the appearance of the rats was much better. Absolute alcohol removed a considerable quantity of a red colored material from Wilson & Company's blood meal, and anhydrous ether removed a smaller quantity of yellowish fatty material. However, the hot-alcohol-extracted and ether-extracted blood meal failed to show any improvement in nutritive value. More thoro extraction of blood-meal samples, with still other extracting solvents, may produce blood meal of greater nutritive value.

DISTRIBUTION OF PROTEINS

The nitrogen in blood is distributed between the plasma and corpuscles, food constituents, and waste products. The chief nitrogenous components of blood plasma are the four proteins, fibrinogen, nucleoprotein, serum globulin, and serum albumin, which constitute about 84 percent of the solids. The chief nitrogenous constituent of the corpuscles is the protein hemoglobin constituting about 92 percent of the solid material of the red corpuscles.

The nitrogenous food constituents consist principally of amino acids from digested protein. Among the known nitrogenous waste products are urea, uric acid, creatin, creatinin, and ammonium salts.

The complete composition of blood and the chemical changes that occur in coagulation and heat treatment in the drying process, no doubt, give to blood meal a wider and different nitrogen distribution than fresh blood, depending somewhat on the source of blood and the method of preparation.

TABLE 9.—Nutritive Value of Extracted Blood Meal for Growth of Rats

Distinguishing feature of blood meal product	Level of intake	Lot receiving diet	Duration on experiment	Average daily food consumption	Gain in body weight	Total intake		Intake per gm. of gain		Gain per gm. of food	Gain per gm. of protein
						Food	Protein	Food	Protein		
	<i>Pct.</i>	<i>No.</i>	<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Commercial pig blood non-extracted	36	44	47	8.1	—1	379.0	144.6
Cold water extracted commercial pig blood	36	66	35	6.7	—5	234.5	89.5
Hot water extracted commercial pig blood	36	65	42	5.5	—4	231.0	88.2
Cold alcohol extracted commercial pig blood	36	67	84	6.4	—5	537.6	205.2
Cold ether extracted commercial pig blood	36	73	63	6.1	—9	384.3	146.7
Unextracted Cleveland Provision Co. blood meal.	36	43	110	6.5	37	714.1	272.5	19.3	7.4	0.05	0.14
Cold alcohol and ether extracted Cleveland Provision Co. blood meal	36	50	110	6.7	95	737.0	281.4	7.8	3.0	.13	.3
Cold alcohol hot water extracted Cleveland Provision Co. blood meal	36	51	110	7.8	56	858.0	327.5	15.3	5.8	.07	.2
Wilson & Company unextracted blood meal	36	92	62	3.6	0	220	84.0
Hot ether extracted Wilson & Co. blood meal....	18	100	63	4.1	—9	1172	236.28
Hot alcohol extracted Wilson & Co. blood meal ...	18	101	49	3.1	—12	612	123.38

The previous part of this work on the digestion, growth-promoting value, and metabolism of blood-meal protein is based on the conception that the protein value is governed by the nitrogen content multiplied by the factor 6.25. The work gives results that answer for practical purposes, but does not throw much light on the distribution of nitrogen or chemical characteristics of the proteins.

The nitrogen of a food may be classed into two general forms, namely, nitrogen existing as protein and non-protein nitrogen.

NON-PROTEIN NITROGEN

The non-protein nitrogenous substances cannot be neglected in assessing the nutritive value of a food as a source of protein, since these substances, consisting so largely of amino acids and amino-acid derivatives, may supplement the true proteins or may even possess a definite value of their own in satisfying the nitrogen of animals. Grindley (32) found only 1.87 percent of the total nitrogen of blood meal existing as non-protein nitrogen. Of this amount, 0.4 percent consisted of forms of nitrogen which result from the decomposition of proteins by hydrolysis.

It was desired (1) to secure further data on the non-protein nitrogen of blood meal; (2) to determine differences in the non-protein nitrogen, lipoid, and fat content of blood meal from different species; and (3) to remove impurities that might hinder subsequent analysis for amino acid distribution.

The non-protein nitrogen was removed by extraction according to the method employed by Hamilton (36). Ten-gram samples, in duplicate, were dried and placed in 500 cc. centrifuge bottles, 100 cc. of anhydrous ether was added, and the samples were shaken for three hours. The samples were then centrifuged and the liquid filtered off. Four similar extractions were made, the fourth being collected separately to determine the relative amount extracted. The ether extractions were followed by similar extractions with absolute alcohol and then with 1 percent trichloroacetic. The small amount of protein dissolved by the trichloroacetic acid solution was precipitated by ferric hydroxide. The reliability of this protein precipitant has been shown by Van Slyke (89). He found that ferric hydrate not only allowed all the amino acids to pass into the filtrate, but that it also precipitated none of the intermediary products up to the albumoses, and none of these except some of complexity but little below that of the original proteins.

TABLE 10.—Non-Protein Nitrogen Content of Blood Meal

Product	No.	Sample		Anhydrous ether extraction	Absolute alcohol extraction	Trichloroacetic acid extraction	Total non-protein nitrogen removed
		Weight	Nitrogen content	Nitrogen removed	Nitrogen removed	Nitrogen removed	
Cleveland Provision Co. blood meal		<i>Gm.</i>	<i>Gm.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
	a	10	1.3446	0.29	0.31	0.31	0.91
	b	10	1.3446	.32	.36	.32	1.00
	Av.31	.33	.32	.96
Wilson & Co. blood meal	a	10	1.2972	.06	.61	.08	.75
	b	10	1.2972	.12	.31	.09	.52
	Av.09	.46	.08	.63
Air-dried sheep blood	a	10	1.3370	.08	.40	.07	.55
	b	10	1.3370	.06	.45	.06	.57
	Av.07	.43	.06	.56
Air-dried pig blood	a	10	1.3536	.10	.68	.05	.83
	b	10	1.3536	.17	.43	.05	.65
	Av.13	.55	.05	.74

The non-protein nitrogen removed by the different solvents is given in Table 10. The fourth extraction by ether removed 0.02 percent of the total nitrogen, 0.07 percent by alcohol, and 0.01 percent by trichloroacetic acid. Altho the extractions were not complete, it was evident that the non-protein nitrogen content was very low. Apparently the trichloroacetic acid removed the least nitrogen and alcohol the most. No appreciable difference was noticed in the blood from different species.

TABLE 11.—Extractives of Blood Meal (Percent of Total)

Blood meal sample	Solids removed by ether	Solids removed by alcohol	Total solids removed	Nitrogen removed by ether	Nitrogen removed by alcohol	Total nitrogen removed
	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
Cleveland Provision Co. . . .	0.83	3.45	4.28	0.76	0.96	1.72
Wilson & Co.28	5.41	5.69	.00	.98	.98
Air-dried sheep blood.67	2.49	3.16	.05	.47	.52
Air-dried pig blood.53	2.77	3.30	.02	.26	.28

Extracted first with ether and then alcohol in Soxhlet extractors for 48 hours.

The total nitrogen and solids, including fats and lipoids, removable by hot extraction with anhydrous ether and alcohol, were determined on a number of blood-meal samples. Ten-gram moisture-free samples were extracted first with ether and then with alcohol in Soxhlet extractors for a period of 48 hours. The results are presented in Table 11. Alcohol dissolved six times as much solid material, and three times as much nitrogen as ether dissolved. More extractives were removable from commercial brands of blood meal than from air-dried samples. The ether extract from all samples was practically colorless, while the alcohol extract from the Wilson & Company product had a dark red color, and the extract from the other samples had a yellowish tinge.

AMINO ACID DISTRIBUTION

All considerations and deductions as to the dietetic value of proteins can no longer be based on total nitrogen content but must be based on the available amounts of the various amino acids. Nitrogen equivalence is not amino-acid equivalence. Proteins devoid of certain units are of no value for the maintenance of life. Proteins deficient in certain amino acids are of little or no value for ensuring the growth of young animals. Then the biological value of a protein is primarily limited by the proportions in which the amino acids that cannot be synthesized by an animal occur in its molecule. This chemical limitation is such that for each protein or mixture of proteins a deficiency in one of the indispensable amino

acids curtails its value for structural purposes in the animal body. When this deficiency is remedied by the addition of the appropriate amino acid, a deficiency of some other amino acid may constitute a second limiting factor, etc.

DISTRIBUTION OF NITROGEN IN THREE GROUPS

Since a complete analysis of a protein is still an impossibility, owing to the unsatisfactory methods for isolating and estimating the several mono-amino acids, the proteins cannot yet be differentiated by means of their chemical composition. It has been shown by Osborne (65) that the methods for estimating the ammonia content and the diamino-acid content of a protein are reliable. Therefore, the nitrogen distribution into four groups was made on a number of samples of blood meal by the Van Slyke (88, 89) method as modified by the methods used by Moulton (61) and Plimmer (74).

The results are presented in Table 12. Similar distribution of nitrogen in blood proteins and casein as found by other investigators are included for comparison. Considerable variation in results is shown. The method of preparation of sample for analysis influences the results obtained. This is shown by the Cleveland Provision Company's blood meal where extraction caused a lower percentage of di-amino nitrogen and a higher percentage of mono-amino nitrogen.

TABLE 12.—Percentage Distribution of Nitrogen in Three Groups

Protein	Amide N	Humin N	Di-amino N	Mono-amino N
Wilson & Co. blood meal	5.47	4.03	40.15	50.35
Air-dried sheep blood	4.44	4.04	36.45	55.07
Air-dried pig blood	5.07	4.36	17.76	72.81
Commercial pig blood	5.14	5.07	35.87	53.92
Clev. Prov. Co. blood meal	4.48	4.77	26.84	63.91
Clev. Prov. Co. blood meal (cold ether, alcohol, and trichloroacetic acid extracted)	5.02	4.02	21.12	69.84
Clev. Prov. Co. blood meal (hot ether and alcohol extracted)	5.13	5.10	20.52	69.25
Blood meal (Grindley, 31)	5.85	3.95	33.63	56.57
Blood meal (Nollau, 64)	6.19	5.69	36.59	51.53
Casein (Van Slyke, 88)	10.31	1.34	22.34	66.01

DISTRIBUTION OF NITROGEN IN SEVEN GROUPS

The Van Slyke method permits of a further differentiation of the units composing the protein molecule into those containing amino groups and those containing nitrogen in heterocyclic combination. Since the method permits of the determination of the distribution of the nitrogen in seven groups, it was used for a further separation of blood-meal nitrogen.

The results of the work are given in Table 13. Analyses by other investigators are also included, as well as the nitrogen distribution in casein. As compared with casein, hydrolized blood meal contains less ammonia, and apparently less lysine. Of the essential amino-acids—tryptophane, tyrosine, lysine, and cystine—which cannot be synthesized in sufficient quantity by the animal, not one can be quantitatively determined with a fair degree of accuracy by the group method of analysis.

4—PROTEIN SUPPLEMENTS

The biological value of the proteins in mixtures of foods is not necessarily the weighted mean of the biological values of the proteins of the individual foods. Each protein may be regarded as consisting of two fractions, one an assortment of amino acids that can be used to replenish or enlarge the supply of nitrogenous substances in the tissues, the other including the remaining amount of the constituent amino acids destined to be de-amized, because it does not contain the complete assortment of amino acids essential for synthesis into complexes required by the tissues. If two or more foods are fed together to a growing animal, those fractions of each that would otherwise be de-amized may together contain a complete assortment of amino acids permitting a part of the combined fractions to be used for synthetic purposes. In such cases, obviously the biological value of the mixture would be greater than the weighted mean biological value of each.

In this part of the work with purified rations the control ration used consisted of casein 18, agar 2, concentrated dried brewery yeast (Northwestern Yeast Company) 6, starch 66, Osborne and Mendel (69) salt mixture 4, crisco 2, cod-liver oil 2. When other protein concentrates were added the starch content was varied so as to keep the total on the basis of 100. Results are reported for the average of three rats in a lot. Selection and management of rats were the same as for growth studies. The Wilson & Company spray-process blood meal was used. The experimental period for each lot was 8 weeks. The data are summarized in Tables 14 and 15 and Charts 3 and 4, and the percentages of the essential amino acids in some of the proteins used are given in Table 16.

Casein supplements blood meal, as shown in Tables 14 and 15 and Chart 3. This supplementary relationship is also shown in Table 8, where the biological value of blood meal was found to be 19, casein 38, and blood meal and casein 42. Table 14 shows that the blood meals from various species of animals when fed with a small

TABLE 13.—Distribution of Nitrogen in Seven Groups

Protein	Amide N	Humin N	Cystine N	Arginine N	Histidine N	Lysine N	Mono-amino N	Non-amino N	Total N
	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
Clev. Prov. Co. blood meal	4.48	4.77	1.85	6.63	13.76	0.03	62.21	7.99	101.72
Blood meal (Grindley, 31)	5.85	3.95	0.69	9.16	8.53	9.73	56.57	4.42	98.90
Blood meal (Nollau, 64)	6.19	5.69	2.02	7.72	8.37	9.97	51.53	3.94	95.44
Casein (Van Slyke, 88)	10.27	1.28	.20	7.41	6.21	10.30	55.81	7.13	98.61
Fibrin (Van Slyke, 88)	8.32	3.17	.99	13.86	4.83	11.51	54.30	2.70	99.68
Hemoglobin (Van Slyke, 88)	5.24	3.60	.00	7.70	12.70	10.90	57.00	2.90	100.04
Tankage (Grindley, 31)	6.58	4.40	1.28	14.15	4.94	7.48	52.39	7.27	98.49
Cottonseed meal (Grindley, 31)	10.45	7.78	.65	19.52	5.47	4.78	42.82	5.43	96.90
Wheat bran (Nollau, 64)	9.67	13.75	5.96	12.53	3.84	4.04	49.95	0.00	99.74
Maize kernel (Nollau, 64)	4.63	7.00	4.06	16.19	4.45	8.53	49.69	0.00	94.55

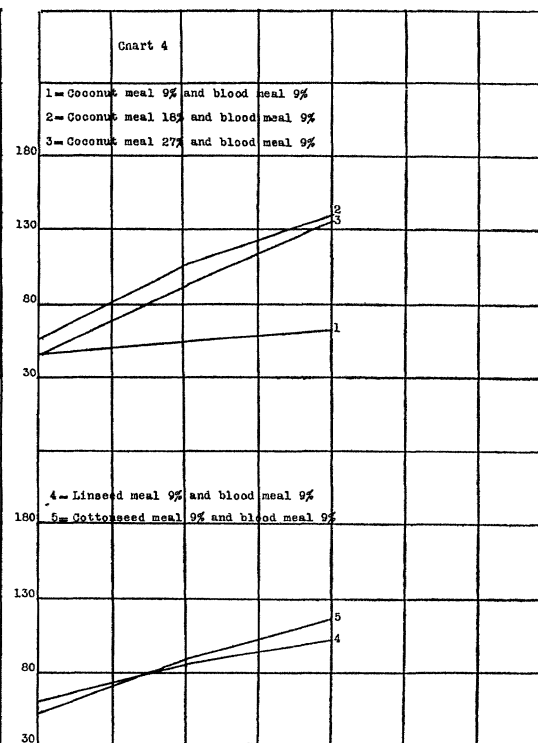
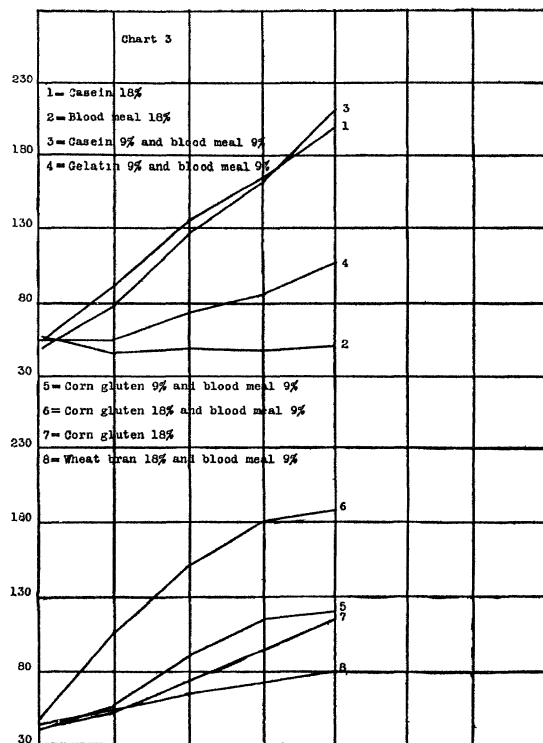
TABLE 14.—Supplements for Blood Meal

Distinguishing features of diet	Lot receiving diet	Duration on experimental diet	Gain in body weight	Average daily food consumption	Gain per gram of food eaten	Food required per gram of gain
	<i>No.</i>	<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Corn.....	10	98	26	7.9	0.03	29.8
Corn and air-dried pig blood.	11	112	59	7.3	.07	13.9
Corn and cooked pig blood.	13	110	60	8.5	.06	15.6
Corn and commercial blood.....	19	112	95	9.5	.09	11.2
Corn and salt mixture.....	52	103	89	10.2	.08	11.8
Corn and air-dried pig blood and salt mixture.....	53	103	184	13.4	.13	7.5
Corn, cooked pig blood, cod-liver oil, and salt mixture.....	54	103	193	6.4	.29	3.4
Corn, commercial blood meal, and cod-liver oil.....	82	49	87	6.4	.28	3.6
Clev. Prov. Co. blood meal, 18 percent, casein 18 percent.....	34	77	181	11.8	.20	5.0
Air-dried pig blood 18 percent and casein 18 percent.....	36	72	186	10.0	.26	3.9
Air-dried sheep blood 18 percent, and casein 18 percent.....	40	79	193	12.0	.20	4.9
Cooked cattle blood 15 percent, and casein 3 percent.....	58	110	88	7.0	.11	8.7
Cooked pig blood 15 percent, and casein 3 percent.....	59	110	124	7.9	.14	7.0
Cooked sheep blood 15 percent, and casein 3 percent.....	62	110	35	4.1	.08	12.9
Wilson & Company blood meal and casein 3 percent....	93	97	69	7.7	.09	10.8

percentage of casein had a difference in nutritive value much the same as when they were fed alone, as shown in Table 6. Casein is one of the most complete proteins, according to Osborne (67), and promotes good growth at a 9-percent level of intake when an additional amount of cystine is added. Then cystine is the first limiting amino acid of casein. Casein showed a marked supplementary

TABLE 15.—Protein Supplements for Blood Meal
(Average of 3 rats for 8 weeks)

Distinguishing characteristic of ration	Food intake	Gain	Feed required for a gram of gain
	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Casein.....	628	142	4.4
Blood meal 18%.....	295	—4
Casein 9% and blood meal 9%.....	596	157	3.8
Casein 9%.....	373	74	5.1
Casein 18% and blood meal 9%.....	661	213	3.1
Dried buttermilk 9% and blood meal 9%.....	405	54	7.5
Dried skim milk 9% and blood meal 9%.....	403	59	6.8
Gelatin 9% and blood meal 9%.....	398	52	7.7
Wheat bran 18% and blood meal 9%.....	309	31	9.9
Wheat bran 9% and blood meal 9%.....	253	12	21.0
Corn gluten 9% and blood meal 9%.....	380	77	4.9
Corn gluten 18% and blood meal 9%.....	523	137	3.8
Corn gluten 18%.....	362	47	7.7
Cocconut meal 9% and blood meal 9%.....	293	17	17.2
Cocconut meal 18% and blood meal 9%.....	484	78	6.2
Cocconut meal 27% and blood meal 9%.....	439	92	4.7
Linseed meal 9% and blood meal 9%.....	352	41	8.6
Cottonseed meal 9% and blood meal 9%.....	391	66	5.9



Charts 3 and 4 showing value of supplements used with blood meal for growth of rats. Vertical spaces represent weight in grams, and each horizontal space represents two weeks experimental period

value for blood meal when fed in the metabolism studies in the proportion of blood meal 15 percent and casein 3 percent. The benefit was probably due to one or more essential amino acids other than cystine, since it constituted a very small percentage of the addition. Also, the analyses given in Table 13 show that the cystine content of blood meal is greater than that of casein. Then the limiting amino acids of blood meal must be among the other six regarded as essential by Sherman (83), namely, arginine, histidine, lysine, proline, tryptophane, and tyrosine.

TABLE 16.—Essential Amino Acid Content of Proteins Used as Blood Meal Supplements

Protein	Arginine	Cystine	Histidine	Lysine	Proline	Tryptophane	Tyrosine
	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
Casein (Sherman, 83)	3.81	0.50	2.84	7.62	7.63	2.20	6.50
Gelatin (Sherman, 83)	8.22	.31	.90	5.92	9.5001
Blood meal (Grindley, 31)	11.31	1.36	14.21	5.28
Maize kernel (Nollau, 64)	16.19	4.06	4.45	8.53
Wheat bran (Nollau, 64)	12.53	5.96	3.84	4.04
Tankage (Grindley, 31)	14.15	1.28	4.94	7.48
Cottonseed meal (Grindley, 31)	19.52	.65	5.47	4.78
Globin of haemoglobin*	5.40	.30	11.00	4.30	2.30	Present	1.3
Serum albumin*	2.50	Present	2.1
Serum globulin*90	2.80	Present	2.5
Fibrin*	1.20	3.60	1.30	3.5

*Plimmer (1917).

It is interesting to note that dried buttermilk and dried skim-milk were of equal value as supplements for blood meal, but not as good as casein. This may be explained by the lower protein intake on the milk rations. Either casein or increasing the percentage of protein in the rations resulted in greater food intake.

Gelatin supplements blood meal, but to a less degree than casein (Chart 3). Dakin (16) and Mendel (54) have shown that cystine, tryptophane, tyrosine, and possibly histidine are the only essential amino acids deficient in this product. The analysis given in Table 13 shows that blood meal carries a greater amount of cystine and histidine than the complete protein casein. Therefore, the first limiting amino acid in blood meal is probably either tryptophane or tyrosine.

Either corn gluten or wheat bran supplements blood meal, as shown in Chart 3. Zein is the principal protein in corn. Osborne (66) showed that corn is deficient in lysine and tryptophane. Therefore, it appears that tyrosine is the first limiting amino acid in blood meal. This agrees with data in Table 16, which shows that casein, which is a good supplement for blood meal, is rich in tyrosine.

Cocoanut meal, linseed meal, and cottonseed meal supplement food meal, as shown in Chart 4. It appears that all of the natural protein concentrates improved the palatability and food consumption of purified rations containing blood meal. Results reported in Table 14 are not directly comparable, since there was a difference in food intake, protein level, and rate of growth. There was a possibility that the vitamin intake rather than the protein was a limiting factor in rations that were unpalatable and that had a low intake. Also, the level of protein intake was too near the optimum to show marked differences in the proteins.

A new series of experiments was conducted to check these findings and to eliminate some of the possible errors. Three rats were used to a ration. They were kept in separate cages. Each rat was made to eat as nearly as possible the same quantity of food. The vitamins were given separate from the food. Cod-liver oil was given by pipette, 1 cc. per rat every other day. Yeast was fed at the rate of 0.4 gram per rat every other day. The control ration consisted of starch 87, casein 8, Osborne and Mendel salt mixture 4, sodium chloride 1. An effort was made to get a somewhat similar food intake in the various lots. Proteins were incorporated in proportions to make the nitrogen content equivalent to that of the casein replaced or used in the control ration. The summary of results is given in Table 17.

TABLE 17.—Protein Supplements for Blood Meal
(Average per rat for 8 weeks)

Distinguishing characteristic of ration	Food intake	Gain	Feed required for a gram of gain
	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Casein 8%	246	28	8.8
Blood meal 9.5%	141	—32
Blood meal 4.7% and casein 4%	191	12	15.9
Blood meal 4.7% and corn gluten 9.4%	196	12	16.3
Blood meal 4.7% and meat meal 5.2%	177	5	35.4
Blood meal 4.7% and cottonseed meal 11.2%	228	1
Blood meal 4.7% and bran 24.6%	203	10	20.3
Blood meal 4.7% and gelatin 4%	183	3	61.0
Casein 12%	291	37	7.9
Casein 6% and blood meal 7.5%	275	26	10.6
Blood meal 14.25%	Lost weight and died
Blood meal 14.25% and crisco 5%	134	—12

The blood meal was much less palatable than casein. When the casein ration intake was limited to that of the blood-meal ration, the rats all died during the third week. It is possible that blood meal is more efficient for maintenance than casein. Casein and corn gluten appeared to be of equal value as supplements to blood meal when fed on the same level of nitrogen intake and in equal quantities. At the higher level of casein intake, the feed

consumption was better and better growth was secured per gram of food intake. Bran, meat meal, gelatin, and cottonseed meal fed with blood meal made the rations more palatable.

Food intake on the blood-meal rations was too low to meet the calorific requirement for maintenance. The addition of 5 percent crisco raised the calorific intake, but left the food intake so low that it was hard to judge the value of the protein consumed. The addition of sugar in one case and butter in another increased the palatability of the blood-meal rations but little.

Altho purified rations may be used to show the nutritive value of a protein, they fail to show its value when fed in practical rations. Blood meal finds its chief use as a feed as a protein concentrate for feeding with cereal grain rations. The same rations fed to pigs in digestion trials were fed to rats for growth studies. The results are shown in Table 14. The rats on the corn diet made little growth, but those on rations supplemented by blood meal grew for a time and then passed into a characteristic mineral deficiency state. The rations were then supplemented with the McCollum (51) salt mixture, and with cod-liver oil. Quite different results were obtained by feeding the modified rations. The salt mixture improved the corn ration, and blood meal improved it still more. With corn fed alone protein was the first limiting factor for growth, then mineral matter. Hogan (43) reported similar results.

Altho the data do not show a quantitative supplementary relationship between corn and blood-meal proteins, they show that a favorable supplementary relationship does exist.

SUMMARY

The first limiting factor of the nutritive value of blood meal is palatability. In general, it is low, but varies depending on source of blood and method of preparation.

The digestibility of blood-meal nitrogen is decreased by increasing the temperature in the process of preparation.

The biological value of blood-meal protein for growth is poor.

The basic amino acids in blood meal occur in approximately the same quantity as in casein.

Casein and corn gluten are efficient protein supplements for blood meal.

Blood meal properly prepared for animal feeding purposes is not toxic.

Swine blood has a higher nutritive value for the rat than cattle or sheep blood.

Blood serum has higher nutritive value than the clot.

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